The Synthesis and DNA Footprinting of Acridine-linked Netropsin and Distamycin Bifunctional Mixed Ligands

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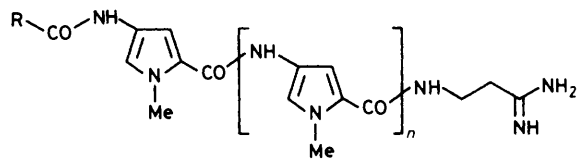
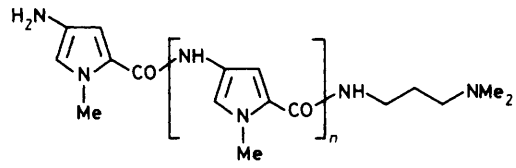
The new synthetic acridine-linked netropsins (**9a—c**) and distamycin (**10a—c**) are bifunctional mixed ligands which bind to DNA by both intercalation and minor-groove association and show enhanced preference for A–T rich sites.

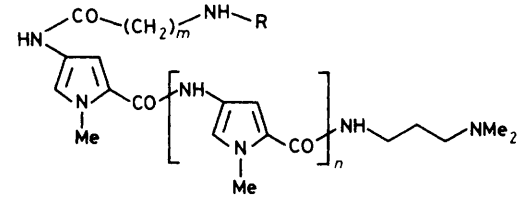
The structurally related antiviral antibiotics netropsin (**1**) and distamycin (**2**) bind to double helical B-DNA along the minor groove. *X*-Ray analysis of a co-crystalline complex of netropsin with a dodecanucleotide duplex has confirmed the nature of the association.[1] Extensive DNA-footprinting and affinity cleavage experiments by Dervan's group[2] have shown that these pyrrole oligopeptides and their higher analogues strongly favour A–T-rich regions of DNA as their binding sites. For example, distamycin binds strongly to the 5′-AATT-3′ site.



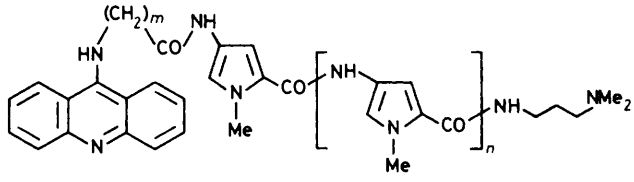
(1) $n = 1$, $R = CH_2NHC(NH)NH_2$
(2) $n = 2$, $R = H$



(3) $n = 1$
(4) $n = 2$



(5) $n = 1$, $R = Cbz$ (7) $n = 1$, $R = H$
(6) $n = 2$, $R = Cbz$ (8) $n = 2$, $R = H$



(9) $n = 1$ (10) $n = 2$
a; $m = 2$ b; $m = 3$ c; $m = 4$

Acridines bind to DNA by a second mode of association known as intercalation.[3] Antibiotics which bind to DNA primarily by intercalation, but also with a contribution from a minor-groove association, include actinomycin,[4] triostin A,[5] and echinomycin.[6] Recently, some synthetic ligands derived from phenoxazone and netropsin/distamycin analogues (distaxins),[7] and a bis[Fe^{II}·EDTA–distamycin]phenoxazone (EDTA = ethylenediaminetetra-acetic acid)[8] were reported to show some evidence of bifunctional binding to DNA. We report here the synthesis, characterization, and DNase I footprinting of a series of bifunctional mixed ligands [(**9a**—**c**) and (**10a**—**c**)] derived from 9-aminoacridine, netropsin, and distamycin, and linked by variable length spacers.

The diamines (**3**) and (**4**) were prepared in ten and twelve steps, respectively,[9] from *N*-methylpyrrole. The imidazolides of the *N*-Cbz (Cbz = benzyloxycarbonyl) protected amino acids, β-alanine, γ-aminobutyric acid, and δ-aminovaleric acid [carbonyldi-imidazolyl (CDI), dimethyl formamide (DMF), 90 min] were condensed in turn with (**3**) and (**4**) to yield (**5a**—**c**) and (**6a**—**c**). Hydrogenolysis (Pd/C, MeOH, 30 min)

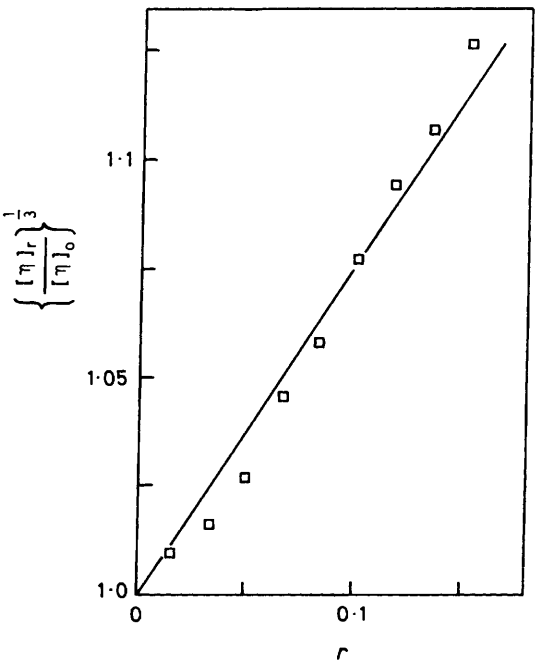


Figure 1. Viscometric titration of sonicated calf thymus DNA (1.0 mM base pairs) with (**10c**) at 20° C, PIPES buffer (ionic strength 0.1 M), pH 6.8. $[\eta]_r$ and $[\eta]_o$ are the intrinsic viscosities in the presence and absence of drug and r is the drug/base-pair ratio.

gave the free amines (**7a**—**c**) and (**8a**—**c**),† which were treated *in situ* with 9-phenoxyacridine to produce the two series of bifunctional mixed ligands (**9a**—**c**)† and (**10a**—**c**)† which were purified by preparative h.p.l.c. [MeOH–H_2O–NH_3 (80 : 19.8 : 0.2), C_{18} reversed phase column].

Viscometric measurements were performed on solutions of sonicated calf thymus DNA in the presence of (**9a**—**c**) and (**10a**—**c**). Control experiments were also carried out with (**7b**) and (**8b**). The fractional increase in the length of the DNA (L/L_o) was correlated to the intrinsic viscosities $[\eta]_r$ and $[\eta]_o$[10] and a typical plot is shown in Figure 1 for (**10c**). All of the compounds (**9a**—**c**) and (**10a**—**c**) caused lengthening of the DNA consistent with intercalation of the acridine moiety. In contrast, (**7b**) and (**8b**) showed no such effect.

The dissociation kinetics of the ligand–DNA complex were studied using the SDS drug sequestration method[11] and the data were analysed by use of the CONSAM curve-fitting program. The dissociation processes for (**9a**—**c**) and (**10a**—**c**) were found to be about 1000 times slower than for 9-aminoacridine, and about ten times slower than for either distamycin or netropsin. This suggests a mode of co-operative binding to DNA and that (**9a**—**c**) and (**10a**—**c**) are behaving as true bifunctional mixed ligands.

A 180-mer restriction fragment (Hind III, Pvu II) from the plasmid pSP65 was 3′-end labelled with [^{32}P]dATP (AMV reverse transcriptase) and isolated by polyacrylamide gel electrophoresis (p.a.g.e.). The labelled 180-mer fragment was incubated with each of the ligands (**9a**—**c**) and (**10a**—**c**) and the controls (**7b**) and (**8b**) in turn, at various ligand/DNA concentrations in the presence of DNase I (25 °C, 2 h). The digestion products were separated (p.a.g.e.) and the resultant autoradiograms were scanned by densitometry. A typical gel is shown in Figure 2 for (**10b**). The square brackets on the left

† Satisfactory ^{1}H and ^{13}C n.m.r. and fast atom bombardment mass spectral data have been obtained.

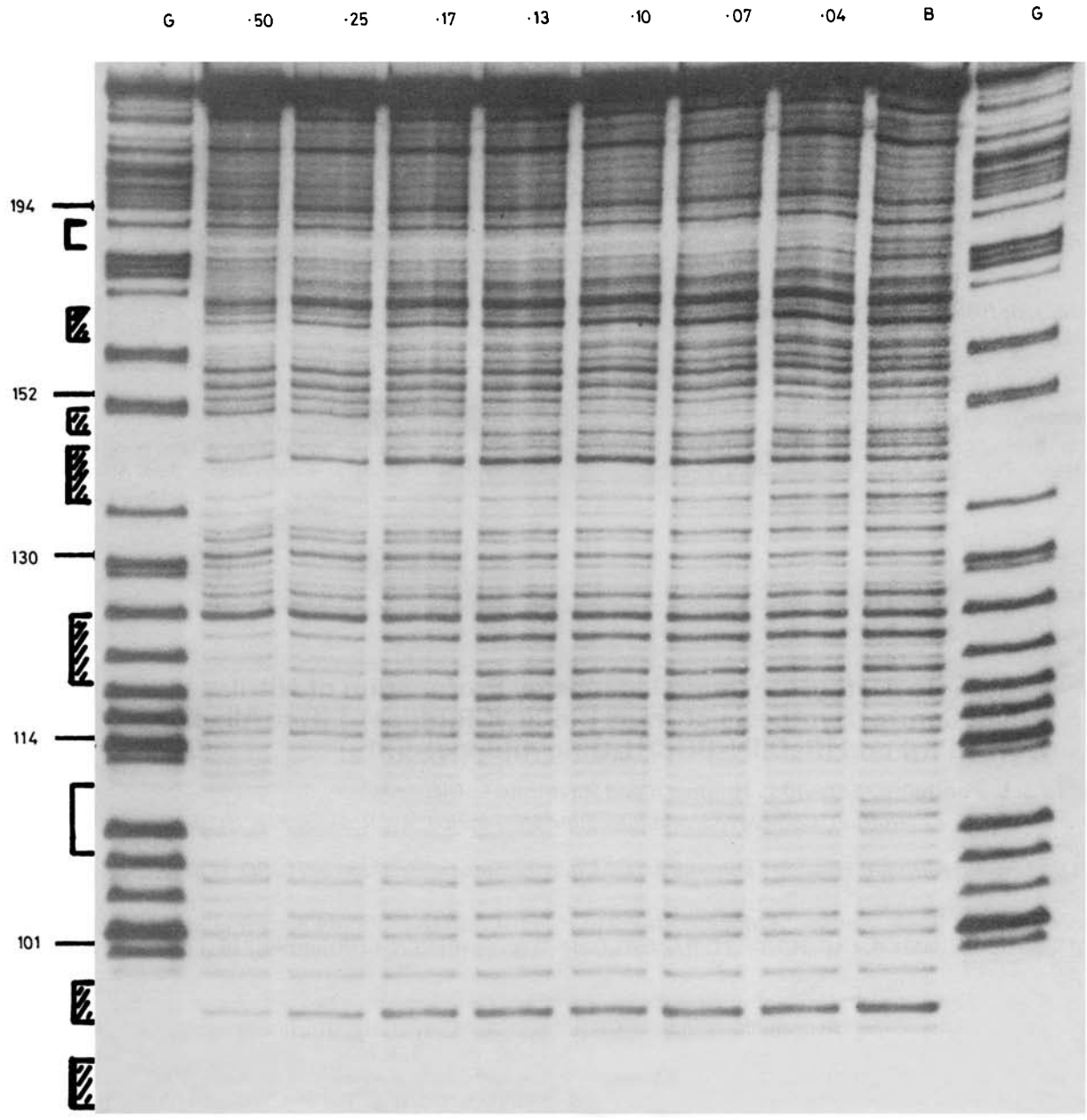


Figure 2. Autoradiogram of a denaturing polyacrylamide gel, ^{32}P 3′-end labelled 180-mer DNA. Lane G: Maxam–Gilbert chemical sequencing G reactions; Lane B (blank): DNase I digest in the absence of ligands; Lanes 0.5—0.04: DNase I digests in the presence of decreasing concentrations of (**10b**) from 0.5 to 0.04 drug/base-pair ratio. The numbered sequence of the restriction fragment is shown at left. The square brackets indicate the areas of protection afforded the DNA by the ligand from digestion by the DNase I. The hatched square brackets show the new areas of protection provided by (**10b**) in addition to those afforded by (**8**).

of Figure 2 show the regions of protection afforded the DNA by ligand (**10b**). In particular, the hatched areas represent the new areas of protection which (**10b**) provides in addition to those afforded by the reference compound (**8**), presumably due to the presence of the acridine moiety in (**10b**).

In general, all the bifunctional mixed ligands (**9a**—**c**) and (**10a**—**c**) showed more binding sites and greater protection to DNase I digestion than the two controls (**7b**) and (**8b**). These controls behaved similarly to netropsin (**1**) and distamycin (**2**).[2] Minor variations were observed in the footprints of (**9a**—**c**) and (**10a**—**c**), reflecting the effect of linker chain length *m*. Corey–Pauling–Koltun models of these compounds suggest that a linker length of $m = 3$ would provide an optimum fit. Typical binding sites were found to be of 5—6 base pairs in size and characteristically, in A–T rich regions. The greater number of observed binding sites is certainly due to the increased DNA affinity of the bifunctional mixed ligands. Larger regions of protection observed on the DNA are probably composites of two or more actual binding sites. Although the presence of the acridine moiety enhances the overall binding of these ligands, it does not appear to alter the preference of the pyrrole–amide residues for A–T rich sites.

The regions of high G–C content in the DNA showing enhanced digestion by DNase I in the presence of (**9a—c**) and (**10a—c**) appear to be due to transmission of a drug-induced conformational effect along the DNA; this effect has previously been observed by others.[12]

We thank the Australian Research Grants Scheme and the Anti-Cancer Council of Victoria for financial support, and Dr. J. A. Edgar, CSIRO for determining the fast atom bombardment mass spectra.

Received, 19th January 1988; Com. 8/00187A

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